Inhibition of Osteoporosis Due to Restricted Food Intake by the Fish Oils DHA and EPA and Perilla Oil in the Rat

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Note

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Seven-week old female rats fed restricted foods including the fish oils Docosahesanoic Acid (DHA) and Eicosapentaenoic Acid (EPA) and perilla oil with food intake decreased by 50%, had increases of fracture force and bone mineral density (BMD) and decreases in levels of Deoxypiridinoline (Dpd) and Calcium (Ca) in the urine, compared with those of rats with osteoporosis due to restricted soy bean oil food intake. Therefore, the fish oils DHA and EPA and perilla oil depressed excretion of urinary Ca and inhibited osteoporosis due to restricted food intake.

Key words: osteoporosis; docosahesanoic acid (DHA); eicosapentaenoic acid (EPA); deoxyxypirinoln; restricted food

Rats fed a restricted diet containing 50% less carbohydrate and oil, but normal levels of protein, minerals, and vitamins, have osteoporosis1–3) with a significant fall in BMD and fracture force and an increase in femoral X ray image density. The feces contain a high concentration of Dpd.4) Osteoporosis due to restricted food intake is similar to type I osteoporosis with respect to fecal Dpd and inhibition by estrogen,1) and exercise.2) Type I appears to be induced by loss of appetite and decrease of food intake due to menopausal symptoms after menopause.

Essential fatty acids of polyunsaturated fatty acid such as linolic acid (18: 2n−6), alpha linolenic acid (18: 3n−3), arachidonic acid (20:4n−4), eicosapentaenoic acid (20: 5n−3, EPA), and docosahexaenoic acid (22: 6n−3, DHA) are necessary to take every day due to the fact that they can not be synthesized in the body.5) They produce eicosanoids of 20 carbon atoms such as PGE1, PGE2, and PGD2.6,7) Loss of PGE2 in the post-menopausal female leads to osteoporosis.8) PGE2 has also been demonstrated to enhance PTH-stimulated bone resorption.9) Linoleic acid, EPA, and gamma linolenic acid supplementation are reported to increase Ca absorption10,11) and decrease calcium excretion.12–15)

Hence inhibition of osteoporosis due to restricted food intake by SB oil rich in linoleic acid, P oil with much alpha linolenic acid, and the fish oils EPA and DHA was investigated in this study.

Standard foods of Group 1 according to the AIN93G formula, SB oil (18: 2n−9 55%, 18: 1n−9 30%, 16: 0 13%), foods of Group 2, P oil (18: 3n−3 60%, 18: 1n−9 20%, 18: 6n−2 15%), foods of group 3, DHA (22: 6n−3 49.5%, 18: 1n−9 10%, 20: 5n−3 9.8%), foods of Group 4, and EPA (20: 5n−3 28.1%, 22: 6n−3 14.1, 18: 1n−3 9.6%, 16: 1n−7 8.2%, 16: 0 8.0%), foods of Group 5 were prepared as indicated in Table 1.

Table 1. Dietary Composition

<table>
<thead>
<tr>
<th></th>
<th>Ad libitum (g/100 g diet)</th>
<th>Food restriction of 50% (g/100 g diet)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>Casen</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>β-Cornstarch</td>
<td>39.7</td>
<td>17.4</td>
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<td>α-Cornstarch</td>
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<td>4.7</td>
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<td>Sucrose</td>
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<td>5.0</td>
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<td>Oil</td>
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<td>14.0</td>
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<tr>
<td>Fiber cellulose (Soybean)</td>
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<td>8.8</td>
</tr>
<tr>
<td>Mineral mix (Soybean)</td>
<td>5.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Vitaminemix (Perilla)</td>
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</tr>
<tr>
<td>t-Cystine</td>
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<td>0.6</td>
</tr>
<tr>
<td>Choline bitatrate</td>
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<td>0.5</td>
</tr>
<tr>
<td>t-Butylhydroquinone</td>
<td>0.0014</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

Groups 1, 2, 5 (n = 5), Groups 3, 4 (n = 6).
The basal composition of experimental diets was based on the AIN-93G (American Institute of Nutrition) formulation.
Standard and Control were compounded to soybean oil.

Footnotes:

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Abbreviations: EPA, eicosapentaenoic Acid; DHA, docosahesanoic acid; PG, prostaglasin; P oil, perilla oil; SB oil, soy bean oil; Dpd, deoxyxypiridinoln; BMD, bone mineral density
The amounts of protein, oil, vitamins, and minerals for Groups 2, 3, 4, and 5 were doubled, as compared with those for Group 1.

Female rats (6 weeks old) of the Wistar strain were kept on standard feed for 1 week before the start of the experiment. The animals were then separated into 5 groups of 5 rats each. Group 1 was fed ad libitum the standard AIN-93G feed. Group 2 was fed SB oil food with food intake deceased to 50%, compared with the amount fed Group 1. Group 3 was fed P oil food with the same food intake as that of Group 2. Group 4 was fed DHA food with half the intake of Group 1. Group 5 was fed EPA food with the same food intake as that of Group 2. The amounts of protein, oil, vitamins, and minerals for Group 1 were planned to agree with those for Groups 2, 3, 4, and 5 at daily food intake. The body weight and food intake of each animal were measured every day. Urine was collected for 3 h between 6 to 9 o’clock in the morning before the day finished the animal experiment to analyze Dpd using the metra DPD EIA kit (Sumitomo Co.) and Ca with the Calcium Test Wako (Wako Co.). The animals were killed after 3 weeks and blood was drawn. The serum from the blood was collected by centrifuging the blood at 12,000 rpm for 20 min to measure concentrations of estradiol and PGE$_2$ using an ELA kit (Funakoshi Co.). The liver, kidney, adrenal gland, and uterus were then removed, and their total weights were measured. The right and left femurs were removed and stored at $-60^\circ$C to estimate fracture force, BMD, and density of X-ray image, as previously described.$^{1–3}$ The Ryukyu University Guidelines for the Care and Use of Laboratory Animals were followed.

Data were tested by one-way analysis of variance followed by inspection of differences between means by Duncan’s new multiple range test throughout these experiments. The different superscript letters in the figures shows statistically significant differences at $p < 0.05$.

The body weight of Group 1 fed standard food was about 120 g at the experiment start and gradually grew to reach 155 g for an increase of 35 g in 3 weeks. The weights for groups 2, 3, 4, and 5 decreased gradually to about 114 g, a fall of about 6 g; 102 g, a fall of 8 g; 109 g, a fall of 11 g; and 111 g, a fall of 9 g respectively. The food intake for Group 1 was about 10 g every day and 212 g in 3 weeks. Those for groups 2, 3, 4, and 5 were 100 g, 100 g, 100 g, and 100 g over three weeks respectively. Heart weights for Groups 2, 3, 4, and 5 were same as for Group 1. The weights of spleen significantly increased for Groups 4 and 5 as compared with that for Group 1. The pancreas weights increased for Groups 3, 4, and 5. The liver values deceased for Groups 2 and 3. The kidney and adrenal gland levels increased for Groups 2, 3, 4, and 5.

The values for the fracture force (Toughness, $10^{-3}$ J) of the femur for Groups 1, 2, 3, 4, and 5 were 32.01 ± 2.66, $^{2}21.81 ± 2.60,^{2}26.37 ± 4.33,^{a}26.60 ± 4.50,^{a}$ and 27.69 ± 3.62$^{a}$ respectively. Group 2, significantly, had low fracture force, compared with that of group 1 rats fed standard food, but Groups 3, 4, and 5 had no significant difference in fracture force. Inhibition of osteoporosis was found for Groups 3, 4, and 5. (Fig. 1)

BMD (g/cm$^2$) values for Groups 1, 2, 3, 4, and 5 were 0.06 ± 0.02, $^{a}0.03 ± 0.00,^{ab}0.03 ± 0.00,^{ab}0.04 ± 0.01,^{ab}$ and 0.04 ± 0.00$^{ab}$ respectively. Groups 5 and 6 had a tendency of inhibition against the osteoporosis.

In density of X-ray image, bone rich in Ca is low, as the photograph of the bone is white because it absorbs X-rays. The density values of the bone X-ray images for Groups 1, 2, 3, 4, and 5 were 0.42 ± 0.06$^{a}, 0.50 ± 0.09^{b}, 0.48 ± 0.10^{a}, 0.50 ± 0.10^{a}$ and 0.43 ± 0.11$^{a}$ respectively.

The levels (pg/ml) of estradiol in serum were 82.92 ± 39.98, $^{b}40.81 ± 6.63,^{b}53.37 ± 23.95,^{b}39.95 ± 15.35,^{b}$ and 40.77 ± 15.40$^{b}$ for Groups 1, 2, 3, 4, and 5 respectively. The values (g/100 g of body weight) of the womb weight were 0.33 ± 0.07, $^{b}0.23 ± 0.04,^{b}0.23 ± 0.04,^{b}0.24 ± 0.03^{b}$ and $0.22 ± 0.08^{b}$ for Groups 1, 2, 3, 4, and 5 respectively. Uterine weights and serum estradiol concentrations for Groups 2, 3, 4, and 5 were significantly lower than that for Group 1. Uterine weight and level of serum estradiol did not appear to be connected with inhibition of osteoporosis.

As for urinary Dpd levels, Groups 2 and 3 had high values compared with that for Group 1, but Groups 4 and 5 had the same values as Group 1. The average Dpd values (nm) were 2179.81 ± 213.20, $^{a}$2570.30 ± 155.50, $^{ab}2663.63 ± 161.80,^{ab}2377.74 ± 134.10,^{a}$ and 2390.55 ± 234.30$^{a}$ for Groups 1, 2, 3, 4, and 5 respectively. The values of Dpd in the urine corresponded well with those of fracture force and BMD for Groups 4 and 5.

![Fig. 1. Fracture Force of Left Femur of Rats.](image)
restricted food intake to decrease Ca excretion.

The values (mg/dl) of urinary Ca were 18.77 ± 5.99, 12.16 ± 4.80, 10.40 ± 3.77, 7.41 ± 1.68, and 7.69 ± 3.06 for Groups 1, 2, 3, 4, and 5 respectively (Fig. 2). Groups 3, 4, and 5, showing inhibition of osteoporosis had low levels. The fish oils DHA and EPA and P oil appeared to inhibit Ca excretion. EPA supplementation is reported to increase Ca absorption and decrease Ca excretion. EPA and DHA might produce other eicosanoids of 20 carbon atom such as PGE2 and PGH2, and DHA, n-3EFA, are reported to be precursors of the 3-series prostanoids and the 5-series leukotrienes, such as LTB4. Hence EPA and DHA might produce other eicosanoids of 20 carbon atom besides PGE2.

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Levels (pg/mg) of PGE2 in serum were 3487.65 ± 75.10, 3387.95 ± 216.08, 3418.03 ± 240.93, 3036.81 ± 295.91 and 3036 ± 295.91 for Groups 1, 2, 3, 4, and 5 respectively. No significant differences were found among them. Polyunsaturated fatty acids are reported to produce eicosanoids of 20 carbon atom such as PGE2 and PGH2. Loss of PGE2 in the post-menopausal female is seen to lead to osteoporosis. DHA, EPA, and alpha linolenic acid did not appear to be connected with production of PGE2. EPA and DHA, n-3EFA, are reported to be precursors of the 3-series prostanoids and the 5-series leukotrienes, such as LTB4. Hence EPA and DHA might produce other eicosanoids of 20 carbon atom besides PGE2.

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References


